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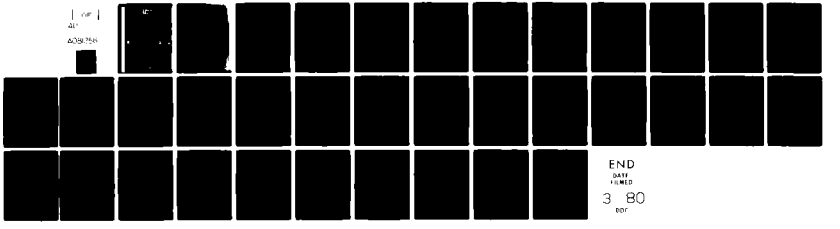

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(6) CANINE AUTOANTICOAGULATION DURING EXTRACORPOREAL PERFUSION

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ABSTRACT

This laboratory has developed a procedure for extracorporeal perfusion without administration of anticoagulants. The present study was designed to determine extra- and intracorporeal requirements for the development of the incoagulable state, to investigate the biologic stability of the perfusion system, and to better define the nature of the hypocoagulable state. Anesthetized dogs were placed on extracorporeal perfusion without exogenous anticoagulation at flows exceeding 800 ml/min. Within 45 minutes of perfusion, blood drawn from the animal was incoagulable (clotting time >24 hr), and within 3 hours following discontinuation of perfusion, clotting time returned to normal. Requirements for the autoanticoagulated state were a perfused liver and a pump in the perfusion circuit. Injections of heparin, 100 U/kg, or use of either Tygon or latex tubing in the perfusion circuit, did not modify the time course of the development of the incoagulable state or its reversal following termination of perfusion. Arterial pressure, heart rate, glucose, platelet and fibrinogen concentrations were relatively constant ($p>0.05$); cortisol, white blood cell and neutrophil concentrations increased ($p<0.05$); while the activity of Factors V, VIII and X decreased ($p<0.05$).

INDEX TERMS: autoanticoagulation, perfusion without anticoagulation, new procedure for perfusion, extracorporeal perfusion, substitutes for exogenous anticoagulation, canine perfusion, improved perfusion, prolonged clotting time, endogenous anticoagulation, endogenous reversal of incoagulability

INTRODUCTION

Exogenously administered anticoagulants have long been considered essential for successful extracorporeal perfusion of man (3,15,16,33) and experimental animals (17,18). However, this anticoagulation, ordinarily obtained by heparin administration, has been associated with adverse side-effects including excessive loss of blood and leakage of fluid from surgical sites (21,33).

Examination of the problems associated with heparinization during extracorporeal perfusion has recently led to the development of perfusion systems that function without the use of exogenous anticoagulants (4,5,11-13). In recent studies employing both venovenous and arteriovenous perfusion systems without exogenous anticoagulation, it was demonstrated that a progressive prolongation of the Lee-White whole blood clotting times (WBCT) occurred until blood became incoagulable (WBCT >2+ hr) after 45 minutes of perfusion (4,5). The animals suffered no adverse acute or chronic effects after 4 to 6 hours of perfusion; normal physiologic status was maintained, tissue pathology was negative; and animals were permanent healthy survivors (4,5). These studies (4,5,11-13) demonstrate that extracorporeal perfusion can be performed utilizing a controlled self-generating hypocoagulable state. A better understanding of this phenomenon may lead to improved management of anticoagulation during extracorporeal perfusion in man.

The purpose of the present study was to better define the intra- and extracorporeal requirements of this type of extracorporeal perfusion; to determine the stability of certain hemodynamic, metabolic, endocrinologic and hematologic parameters during and following perfusion, and to better characterize the autoanticoagulation phenomenon.

METHODS

Experimental Groups

Experiments were conducted on fifty adult dogs divided into four groups: Group 1 was composed of 17 perfused animals administered no exogenous anticoagulant; Group 2 was comprised of 12 dogs administered heparin, 100 U/kg, with six perfused and six not perfused serving as controls. Group 3 was composed of 12 eviscerated dogs perfused without exogenous anticoagulation, six with hepatic artery patent and six with hepatic artery ligated. Group 4 animals (N=9) were eviscerated, endotoxin was administered, and presence or absence of portal hypertension was used as an "in vivo" bioassay of possible collateral hepatic blood flow.

Selection of Animals

Animals selected for these experiments were adult mongrel dogs of random sex, screened for microfilaria, treated for intestinal parasites and allowed to stabilize 3 to 4 weeks. Only animals with white blood cell concentrations less than 21,000/mm^3 and hematocrits exceeding 36% were utilized in the studies.

Surgical and Perfusion Procedures

Intact or eviscerated (see below) animals fasted overnight were prepared for extracorporeal perfusion after receiving intravenous sodium pentobarbital, 25 mg/kg body weight. The right brachial artery of each dog was cannulated to monitor mean systemic arterial pressure (MSAP) and heart rate and for sampling of blood. The basic extracorporeal system consisted of large-bore plastic tubing and plastic reservoir filled with saline (total volume of system about 500 ml). The proximal ends of the first set of saline-filled tubing (clamped with hemostats to prevent saline loss and blood reflux) were advanced into the distal aorta via the left and right femoral arteries.

The distal ends were secured below the surface of a 400-ml saline-filled reservoir placed within a water bath maintained at 40-42°C. Saline-filled tubing, attached to the bottom of the reservoir, was threaded through a roller-type pump and divided into a second set of cannulas which were then surgically inserted into the right and left femoral veins (these lines were also clamped with hemostats to prevent saline loss and blood reflux). Minimal pump occlusiveness was achieved by an adjustment of roller compression so that flow would not occur through the pump in the presence of a hydrostatic pressure gradient of 30 cm H_2O. To initiate the perfusion, all four clamps were simultaneously released with the pump set at a fixed flow rate (range, 800-1200 ml/min), and screw clamps on the arterial outflow lines were adjusted as necessary to maintain a constant level of blood in the reservoir. The pump flow rate was never varied, once established, during each experiment. In a separate series, rubber (latex) tubing was substituted for plastic (Tygon), and the reservoir was removed (see "Results" section).

Animals were eviscerated as follows: after midline laparatomy, the celiac, superior and inferior mesenteric arteries were ligated and divided between ligatures and the following procedure was carried out as previously described (1,17). The intestinal tract was doubly ligated and divided between ligatures at the distal esophagus subdiaphragmatically and at the distal rectum. Following ligation of the portal vein, all abdominal viscera except the liver were surgically removed from the animal, extreme care taken to prevent loss of blood. In half of the experiments, the hepatic artery was maintained patent by sectioning the celiac artery distal to the origin of the hepatic artery; in the other half, the celiac artery was divided near its origin at the aorta, thus preventing flow via the hepatic artery to the liver. Since the portal vein was ligated and sectioned, the liver was without blood flow

in the latter half of the experiments. Eviscerations were completed within 30 minutes, followed by a 30-minute equilibration period.

To confirm that hepatic blood flow was blocked by hepatic artery and portal vein ligation, we administered endotoxin to a series of animals. If portal pressure failed to increase, inflow of blood to hepatic tissue would be considered absent (17).

Prior to, and during, the perfusion period, no animals in Groups 1, 3 and 4 received an anticoagulant of any kind. All catheters and pressure transducers were filled and flushed with nonheparinized saline, and new tubing flushed thoroughly with saline was regularly employed in the perfusion circuit. Preperfusion parameters and blood samples were taken just prior to initiation of perfusion following an equilibration period.

Measurements: General Procedures

The following parameters were recorded in most experiments: mean systemic arterial pressure (MSAP), heart rate, rectal temperature, hematocrit, blood glucose concentration, extracorporeal blood flow rate, platelet and leukocyte concentrations. Coagulation tests included modified Lee-White whole blood clotting time (WBCT), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen concentration, Factors V, VIII and X activities, fibrinogen degradation product (FDP) concentrations, and platelet aggregation to adenosine diphosphate (ADP) and thrombin.

MSAP and heart rate were monitored using a four-channel Hewlett-Packard recorder. Rectal temperature was obtained employing a Tele-Thermometer probe (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). Hematocrit was measured with microhematocrit capillary tubes (Dade Diagnostics, Inc., Miami, Fla.) spun in an International micro-capillary centrifuge (International Equipment Co., Needham Heights, Mass). Blood glucose concentrations

were determined using a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Blood flow was monitored via a calibrated roller-type pump (Medical Specialty Co., Fort Worth, TX) with periodic comparisons using a direct-timed volumetric procedure (see "Methods" section, *Perfusion Procedure*, for details). Platelets were counted using a method previously described (7). White blood cell (WBC) concentrations were obtained from a Coulter automatic particle counter (Coulter Electronics, Inc., Hialeah, FL) and WBC differential evaluations were conducted by staining blood smears with Wright's stain followed by examination with light microscopy. Serum cortisol was determined by radioimmunoassay according to a published procedure (8,22).

Measurements: Coagulation Parameters

Preparation of blood and plasma. Blood used to established normal coagulation control values was drawn from fasted beagle dogs from the external jugular vein using a two-syringe technique. Blood was drawn from the experimental animal's right brachial artery via polyethylene tubing. Both control and experimental blood samples were anticoagulated in Falcon plastic culture tubes using 3.8% sodium citrate (9 parts blood to 1 part anticoagulant).

Platelet-poor plasma was obtained by immediate centrifugation of citrated whole blood at 3000 g for 15 minutes and stored at -20°C in plastic culture tubes until coagulation tests were performed. Platelet-rich plasma used in platelet aggregation studies, performed on the day of each experiment, was prepared from citrated whole blood by centrifugation at 1000 g for 60 seconds in plastic culture tubes (Falcon). Platelet counts were performed using a Coulter automatic particle counter and platelet morphology checked by phase contrast microscopy.

Modified Lee-White whole blood clotting time. One cubic centimeter aliquots of whole blood drawn from the brachial artery were delivered

immediately into three 12x75 mm glass tubes. The tubes remained at room temperature and the first tube was tilted at intervals of 30 seconds until a solid clot formed. The second and third tubes were treated similarly in sequence. The recorded clotting time was the time required for a solid clot to form in the third tube (25).

Prothrombin time (PT). The one-stage prothrombin time was performed (37). First, 0.2 ml of a tissue thromboplastin (rabbit brain and lung) and $CaCl_2$ (0.0125 M) reagent (Simplastin; General Diagnostics, Morris Plains, N.J.) was heated to 37°C for 5 minutes in a plastic test vial. Then 0.1 ml of test plasma was added and a clotting time end point determined with a mechanical device (Clotek-Hyland Laboratories, Los Angeles, CA). The test was performed in triplicate and the mean of the three clotting times was recorded.

Activated partial thromboplastin time (APTT). The APTT was performed as described by Proctor and Rapaport (34). Test plasma (0.1 ml) plus 0.1 ml of a mixture of phospholipid emulsion from rabbit brain and micronized silica (General Diagnostics) was incubated at 37°C for 5 minutes. Then 0.1 ml of 0.025 M $CaCl_2$ was added and the clotting time end point determined with a mechanical device (Clotek-Hyland Laboratories). Test samples were run in triplicate and the mean result was recorded.

Fibrinogen concentration. The fibrinogen levels were determined by procedures described by Clauss (9). A clotting time was performed at 37°C by adding 0.1 ml of thrombin (bovine, 100 U/ml; Dade Diagnostics) to 0.2 ml test plasma (diluted 1:10 with barbital-buffered saline). A standard curve was made using a commercially prepared fibrinogen solution (Data-Fi; Dade Diagnostics) diluted with barbital-buffered saline. Clotting times were performed in duplicate and the mean value was used to determine the fibrinogen concentration from the standard curve.

Platelet aggregation. Citrated platelet-rich plasma (PRP) was stirred in a cuvette and transmittance of incident light, relative to a platelet-poor plasma (PPP) blank prepared from the same blood sample, was recorded using a Chronolog aggregometer (Chronolog, Havertown, PA). Aggregating agents, adenosine diphosphate (ADP) (Sigma Chemical Co., St. Louis, MO) and topical bovine thrombin (Parke-Davis & Co., Detroit, Mich.) were added to the PRP in final concentrations appropriate to produce reversible and irreversible aggregation. Increases in transmittance were used as an index of platelet aggregation (6,31).

Factor assays (V, VIII, X). A modified one-stage prothrombin time was used to measure Factor V (26) and Factor X (2) using human factor-deficient plasma (George King Bio-Medical, Overland Park, KS). A modified activated partial thromboplastin time was utilized to measure Factor VIII (24) levels using human factor VIII-deficient plasma (George King Bio-Medical). A standard curve was constructed using normal pooled citrated canine plasma and the factor-deficient plasma. Three dilutions of the test sample were assayed and a mean value was expressed as a percent of normal activity.

Fibrinogen degradation products (FDP). Fibrinogen degradation products were ascertained using the Thrombo-Wellcotest rapid latex test (Wellcome Research Laboratories, Beckenham, England). Antisera to purified human fibrinogen fragments D and E coat the latex particles, and FDPs in test serum cause the latex particles to clump. Serial dilutions of the test serum were then used to give semiquantitative results. The human antisera cross-react with canine FDPs allowing an estimate of levels to be made in comparison to normal (27,29).

Data Analysis

Data were analyzed using the student "t" test. Only values of less than 0.05 were considered statistically significant.

RESULTS

Table 1 describes various responses of a total of 11 animals to 3 hours of extracorporeal perfusion without exogenously administered anticoagulant, followed by 3 hours of recovery after cessation of perfusion. Lee-White whole blood clotting time increased from a control of 4 minutes to 21 minutes after 30 minutes of perfusion, and by 45 minutes blood removed for Lee-White clotting procedures failed to clot for a period greater than 24 hours (termed "infinite clotting time"). Following cessation of perfusion, clotting time returned to normal within 3 hours. Mean systemic arterial pressure (MSAP), heart rate, blood glucose, fibrinogen and platelet concentrations, prothrombin times, and rectal temperatures (T$_R$) were relatively constant during both 3 hours of perfusion and 3 hours of recovery, indicative of the stability of the preparation.

Striking changes indicated in Table 1 were seen in the following parameters. Cortisol concentrations increased during both perfusion and post-perfusion periods ($p<0.05$), while white blood cell and neutrophil concentrations rose significantly during the post-perfusion period. Activities of Factors V, VIII and X fell markedly during perfusion ($p<0.05$) but were insignificantly different from control values 3 hours after discontinuation of perfusion. The PT and PTT increased during perfusion in all animals; however, the changes failed to reach statistical significance due to the variability of the response.

Table 2 illustrates the effects of extracorporeal perfusion without exogenous anticoagulation on the ability of the platelets to aggregate and on the production of fibrinogen degradation products (FDP) in six of the dogs included in Table 1. On the average, decreased sensitivity to platelet aggregation induced by ADP or thrombin was seen during the 3-hour period of perfusion, while at termination of the recovery period (sixth hour), aggregation

was returning toward the normal (control). FDP levels during and following perfusion ranged from negative at five dilutions to a maximum of positive up to eighty dilutions.

The roles of both "extracorporeal" and "intracorporeal" components in the development of incoagulability are summarized in Table 3. Both Tygon (plastic) and latex (rubber) tubing or the presence of a reservoir had no effect on the development of "infinite clotting time", which was achieved in both instances by 45 minutes with or without a reservoir. Parallel experiments demonstrated that incoagulability of the blood in the absence of a reservoir could be obtained with as little as 33 inches total length of perfusion tubing with a volume of 45 ml; however, the presence of a pump was a requirement. Table 3 shows that recovery characteristics are essentially identical with latex or Tygon tubing, each requiring 3 hours following perfusion for return of clotting times to normal. All 11 animals recoved quickly and were euthanized 7 days after experimentation.

The evisceration studies with and without the hepatic artery ligated demonstrated that a functioning liver was required for the development of "infinite clotting time", but the remainder of the abdominal viscera was not necessary. Clotting time rose slightly, from 5 to 14 minutes in eviscerated dogs with hepatic artery ligation, but the subsequent pattern of autoanticoagulation after 30 minutes occurred only in those animals with the hepatic artery patent. In two of the studies in which the hepatic artery was ligated, clots were observed in the perfusion circuit. One of these animals died during the perfusion.

Data in Figure 1 suggest, however, that hepatic artery patency in eviscerated dogs does not influence MSAP and white blood cell and platelet

concentrations. MSAP fell gradually during perfusion; WBC concentrations fell early but returned to normal after 2 hours of perfusion, rising sharply above control during the 2-hour recovery period; and platelet concentrations showed a similar early trend, but otherwise remained relatively constant (range, 150-250,000/mm^3).

To confirm that hepatic blood flow was completely blocked in the eviscerated dogs with both hepatic artery and portal vein ligations, a series was conducted using endotoxin as a test substance: Table 4 shows that a patent hepatic artery is essential for the development of portal hypertension after endotoxin. These experiments, therefore, provided reasonable assurance that hepatic artery and portal vein ligations in eviscerated dogs prevented blood from entering the liver, thus effectively removing it from the circulation.

Studies were conducted to determine if a low dose of heparin, administered prior to extracorporeal perfusion, would modify the autoanticoagulatory pattern illustrated in Table 1. Figure 2 provides data comparing three types of experimental maneuvers: non-perfused dogs given 100 U/kg heparin intravenously, perfused animals administered the same dosage, and perfused animals receiving no heparin.

Several significant observations emerged from these studies: (*a*) during the first 15 minutes of extracorporeal perfusion, both perfused groups demonstrated depressed clotting times compared to the non-perfused control ($p<0.05$); (*b*) by 45 minutes, blood became incoagulable in both perfused groups, while clotting time was decaying in the non-perfused animals; and (*c*) prior heparinization at low dosages is without influence on the pattern and rate of development of the incoagulable state.

DISCUSSION

This laboratory has developed an extracorporeal perfusion system which functions successfully without the addition of an anticoagulant (4,5). During perfusion a hypocoagulable state is induced. No adverse effects on dogs subjected to 4 to 6 hours of perfusion have been noted, tissue pathology is negative, and animals are permanent healthy survivors.

The purpose of the present study was to advance our understanding of this unique perfusion procedure by identifying extra- and intracorporeal requirements; reaffirming the physiologic stability of the preparation; and further defining the nature of the hypocoagulable state.

Extra- and Intracorporeal Requirements

We have previously reported a procedural requirement that the tubing be saline-filled at the onset of perfusion and that blood flow rates in the extracorporeal system be maintained in excess of 400 ml/min (4,5). A major requirement of the perfusion apparatus was identified in this study. A pump was found to be necessary for the attainment of successful perfusion in the absence of an anticoagulant. Attempts to sustain a stable extracorporeal perfusion system without a pump in the perfusion circuit failed as evidenced by the precipitation of intravascular coagulation and death. The particular pump successfully employed in our studies is relatively atraumatic to the erythrocytes since there is no visible hemolysis after 6 hours of perfusion. Sublethal injuries precipitated by pump trauma to red and white blood cells and platelets undoubtedly occur, however, which might exert subtle effects on cellular function (39).

The type of tubing was not found to be a requirement for the development of extended clotting times in the present study since both latex (rubber) and Tygon (plastic) tubing used in the system produced similar patterns of incoagulability.

Since activation of the coagulation system might be another requirement for the promotion of the hypocoagulable state, heparin was given at the onset of perfusion. From these studies we observed that low doses of heparin (100 U/kg) given prior to perfusion (maximum clotting time <100 min) had no influence on the attainment of "infinite clotting times" (>24 hr) by 45 minutes of perfusion. However, it was noted that the clotting time rose less rapidly in perfused animals following this dose of heparin than in non-perfused controls given a similar dose of heparin ($p<0.05$). This observation suggests that there is an early (15 min) hypercoagulable state, or heparin-resistant state, in the perfused animals. Another explanation, however, is that the concentration of heparin in the blood of perfused animals is decreased due to hemodilution by the saline in the extracorporeal system. The small changes in hematocrit after perfusion, however, indicate that the hemodilution is not great. In any event, this dosage of heparin did not interfere with, or accelerate, the development of the incoagulable state, although its employment might make it possible to use this procedure in humans by insuring that intravascular coagulation would not occur prior to the development of incoagulability.

Intracorporeal requirements were studied to determine essential organ systems influential in the development of autoanticoagulation. It was observed that a perfused liver was required for the development of the incoagulable state. Furthermore, if hepatic perfusion was present, all other abdominal visceral organs did not appear to be necessary in the development of, or recovery from, the hypocoagulable state. The role of the liver is not known; possibilities are that it is essential for the production of an intermediate factor which contributes to the development of incoagulability, or that it may be necessary for the removal of procoagulants.

Stability of Preparation

Consistent with data previously reported by our laboratory (4,5), animals in this study tolerated the extracorporeal perfusion without added heparin. The stability of the preparation is indicated by the relatively unchanging levels of mean arterial pressure, heart rate, blood glucose, fibrinogen and platelet concentrations. We believe the system exerts a mild stress on the animal since plasma cortisol concentrations increased during perfusion and subsequent elevations in white blood cell and neutrophil concentrations occurred during the recovery period. As previously reported (4), all non-eviscerated animals perfused on our system were permanent healthy survivors.

Characterization of the Autoanticoagulated State

Previous investigators have perfused dogs on extracorporeal systems containing blood oxygenators without administering heparin (11-13,32). In these earlier studies, the fibrinogen level (11-13,32) and platelet count (11,13) decreased and the PT, PTT, FDP (11-13) and WBCT (11,12) increased. These changes in the coagulation tests are also reported during disseminated intravascular coagulation (28) with an associated activation of the fibrinolytic system (10).

Our group has reported that dogs perfused using an arteriovenous extracorporeal system with no oxygenator present in the circuit developed WBCT values greater than 24 hours (4,5). This autoanticoagulated state has been further characterized in the current study. Dogs perfused on our extracorporeal system without heparin administration developed a progressive increase in the whole blood clotting time associated with decreases in Factors V, VIII and X activities. The platelet and fibrinogen levels remained relatively stable.

Why is the blood in this system incoagulable? One theory is that the animal is experiencing disseminated intravascular coagulation (DIC). Decreases in Factors V, VIII and X activities similar to those observed in this study have been reported by some investigators after cardiopulmonary bypass performed with heparin administration in man (21) and associated with DIC in man (28) and the dog (14). However, the platelet and fibrinogen concentrations which usually decrease during intravascular coagulation (14,28) did not significantly change in the present study, suggesting that another process (besides DIC) may be responsible for the state of autoanticoagulation.

The elevations of FDP values observed during our perfusion indicate that the fibrinolytic system is activated (10). It is unlikely, however, that this is the primary cause of the incoagulability. Good hemostasis was maintained at surgical sites throughout the study. In addition, the expected decrease in fibrinogen levels reported when fibrinolysis produces a hypocoagulable state (10,23) was not observed in our study. The levels of fibrinogen in our system, however, might not reflect the degree of fibrinolysis if fibrinogen production were accelerated.

A phenomenon which may have contributed to the prolongation of the whole blood clotting time was a change in the reactivity of the platelets. Platelet sensitivity to ADP and thrombin-induced aggregation decreased during perfusion in our experiments. Previous workers have also noted a decrease in ADP-induced aggregation after cardiopulmonary bypass, but the reason is not understood (21).

We have discovered that a plasma inhibitor is generated during the perfusion (see companion paper by Murphy et al., ref. 30). Fibrinogen degradation product concentrations (23) and endogenous heparin (19,20) are inhibitors which have been found in dog plasma in certain experimental situations and either could

theoretically be the major cause of the state of autoanticoagulation produced with our perfusion system. However, the inhibitor we have discovered has heparin-like characteristics in that it accelerates the inhibition of thrombin and Factor Xa by antithrombin III, is heat-stable, and is adsorbed from plasma with $BaSO_4$ (see companion paper, 30).

The inhibitor with heparin-like activity produced in this extracorporeal perfusion system without exogenous anticoagulation is described further in a companion paper (30). Heparin is known to prolong whole blood clotting time and PT and PTT values, inhibit thrombin- and possibly ADP-induced platelet aggregation (38), and at high levels heparin may interfere with the clotting assays used to measure Factors V, VIII and X. Consequently, the elaboration of a heparin-like substance would seem to explain many of the findings in the present study.

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TABLE 1. Response of intact dogs to extracorporeal perfusion without exogenous anticoagulation (Group 1)

		Time (min)										
		Perfusion								Perfusion discontinued		
Parameter	N	0[a]	15	30	45	60	90	120	180	240	300	360
Lee-White whole blood clotting time (min)	11	4.3 (0.3)	9.9 (0.9)*	21.2 (1.6)*	∞ *	∞ *	∞ *	∞ *	∞ *	68.5 (9.1)*	19.7 (1.4)*	5.4 (0.7)
MSAP (mmHg)	11	139 (4)	133 (6)*	127 (6)*	126 (5)*	124 (6)*	124 (7)*	126 (6)*	131 (5)	137 (3)	131 (5)*	126 (4)
Heart rate (/min)	11	158 (11)	186 (7)*	177 (7)*	178 (6)*	172 (6)	172 (8)	177 (6)	167 (6)	146 (9)	170 (5)	175 (10)
Hematocrit (vol %)	11	40.4 (1.2)	37.7 (1.3)*	37.6 (1.6)*	37.5 (1.5)*	38.4 (1.6)*	38.7 (1.7)	39.3 (1.5)	37.7 (1.3)*	37.5 (1.0)*	38.5 (1.0)*	39.0 (0.9)
Rectal temperature (°C)	11	38.6 (0.2)	38.5 (0.2)	38.4 (0.2)	38.5 (0.1)	38.5 (0.1)	38.5 (0.1)	38.6 (0.1)	38.7 (0.1)	39.3 (0.2)*	39.6 (0.2)*	39.7 (0.2)*
Extracorporeal blood flow (ml/min)	11		864 (78)	873 (77)	867 (78)	863 (79)	864 (78)	869 (80)	875 (80)			
Blood glucose (mg/dl)	11	121 (2)	121 (2)	121 (3)	120 (3)	126 (3)	122 (4)	120 (3)	117 (3)	118 (2)	113 (3)	115 (4)
Serum cortisol (mg/ml)	8	154 (42)	209 (40)*				206 (24)		235 (26)*	249 (34)*	254 (47)*	198 (33)
Total WBC (/mm^3)	11	10363 (1190)					6127 (750)*		8936 (1332)	13909 (2093)*	17800 (2610)*	21173 (2919)*
Mature neutrophils (/mm^3)	10	6817 (1041)					4406 (696)*		7114 (1409)	11359 (2315)*	14880 (2830)*	18024 (3207)*
Immature neutrophils (/mm^3)	10	58 (29)					308 (159)		615 (235)*	1179 (400)*	1676 (477)*	2051 (468)*

ble 1 (continued)

		0	15	30	60	90	120	180	240	300	360
Fibrinogen (mg/dl)	6	340 (64)	358 (76)		338 (58)			330 (49)			375 (58)
Platelets ($x10^3/mm^3$)	10	211.2 (29.8)	184.4 (20.8)			197.8 (20.9)		183.1 (21.1)	169.0 (15.4)*		182.2 (20.7)
Factor VIII (% activity)	6	101.7 (1.1)	32.7 (20.3)*		24.2 (8.9)*			14.2 (8.3)*			75.5 (20.2)
Factor V (% activity)	6	99.8 (1.1)	55.4 (12.7)*		66.5 (18.2)			64.8 (17.0)			108.8 (28.0)
Factor X (% activity)	6	99.7 (1.0)	48.4 (13.8)*		43.2 (7.1)*			40.2 (11.3)*			82.8 (16.5)
Prothrombin time (sec)	6	7.4 (0.4)	9.6 (1.5)		11.1 (2.1)			8.6 (0.7)			7.5 (0.3)
Activated partial thromboplastin time (sec)	6	20.3 (1.0)	73.0 (18.6)*		107.0 (35.9)			72.9 (35.8)			24.2 (2.0)

alues are mean(±SE). ∞ = no visible clotting during 24 hours. *p<0.05, paired comparison to 0 time value.

asurements taken prior to beginning perfusion.

TABLE 2. Effects of extracorporeal perfusion without exogenous anticoagulation on platelet aggregation and fibrinogen degradation products (FDP) in intact dogs (Group 1)

	Time (minutes)														
	Perfusion												Perfusion discontinued		
	0[a]			15			60			180			360		
	NA[b]	RA[b]	IRA[b]	NA	RA	IRA	NA	RA	IRA	NA	RA	IRA	NA	RA	IRA
Platelet aggregation[c]															
Adenosine diphosphate (ADP); (μmoles)		2.6 (0.6)	6.0 (1.0)		3.2 (0.7)	7.0 (1.2)		4.4 (0.6)	9.0 (1.0)		5.8 (1.8)	12.0 (3.4)		4.7 (1.9)	10.0 (2.7)
N		5[d]	5		5	5		5	5		5	5		4	5
Platelet aggregation[e]															
Thrombin (units/ml) 1			0.4		2.0	4.0	4.0		8.0		4.0	8.0		2.0	4.0
2	*f*	*f*	*f*	2.0	8.0		4.0		8.0		4.0	8.0			0.4
3			0.4	4.0		8.0	4.0		8.0	2.0		4.0			0.4
4			0.4	8.0			8.0			8.0			0.4		2.0
5			0.4	8.0			8.0			8.0			0.4		4.0
6		0.4	2.0	4.0	8.0		8.0			8.0			0.4		2.0

	Time (minutes)				
	0	15	60	180	360
FDP[g] (dilutions)[h]	<5	>5<80	>5<40	>5<40	>5<20

[a] Measurements taken prior to beginning perfusion.

[b] NA = no aggregation; RA = reversible aggregation; IRA = irreversible aggregation

[c] Values are mean(±SE); final concentration in μmoles.

[d] One animal excluded based on the fact that at 0 time (before perfusion), 20 μmoles of ADP was necessary to aggregate platelets irreversibly.

[e] Values given are individual experiments (1-6).

[f] Measurement not taken. [g] fibrinogen degradation products. [h] Range of values.

TABLE 2. Effects of extracorporeal perfusion without exogenous anticoagulation on platelet aggregation and fibrinogen degradation products (FDP) in intact dogs (Group 1)

	Time (minutes)														
	Perfusion												Perfusion discontinued		
	0[a]			15			60			180			360		
	NA[b]	RA[b]	IRA[b]	NA	RA	IRA	NA	RA	IRA	NA	RA	IRA	NA	RA	IRA
Platelet aggregation[c]															
Adenosine diphosphate (ADP); (μmoles)		2.6 (0.6)	6.0 (1.0)		3.2 (0.7)	7.0 (1.2)		4.4 (0.6)	9.0 (1.0)		5.8 (1.8)	12.0 (3.4)		4.7 (1.9)	10.0 (2.7)
N		5[d]	5		5	5		5	5		5	5		4	5
Platelet aggregation[e]															
Thrombin (units/ml) 1			0.4		2.0	4.0	4.0		8.0		4.0	8.0		2.0	4.0
2	*f*	*f*	*f*	2.0	8.0		4.0		8.0		4.0	8.0			0.4
3			0.4	4.0		8.0	4.0		8.0	2.0		4.0			0.4
4			0.4	8.0			8.0			8.0			0.4		2.0
5			0.4	8.0			8.0			8.0			0.4		4.0
6		0.4	2.0	4.0	8.0		8.0			8.0			0.4		2.0

	Time (minutes)				
	0	15	60	180	360
FDP[g] (dilutions)[h]	<5	>5<80	>5<40	>5<40	>5<20

[a] Measurements taken prior to beginning perfusion.

[b] NA = no aggregation; RA = reversible aggregation; IRA = irreversible aggregation

[c] Values are mean(±SE); final concentration in μmoles.

[d] One animal excluded based on the fact that at 0 time (before perfusion), 20 μmoles of ADP was necessary to aggregate platelets irreversibly.

[e] Values given are individual experiments (1-6).

[f] Measurement not taken. [g] Fibrinogen degradation products. [h] Range of values.

TABLE 3. Effect of extracorporeal perfusion without exogenous anticoagulation on clotting time (in minutes)[a] in intact and eviscerated dogs (Groups 1 and 3)

		Time (minutes)												
		Perfusion								Perfusion discontin				
	N	0[b]	15	30	45	60	90	120	180	210	240	300	330	3
ntact perfused dogs (Tygon tubing and reservoir)	11	4.3 (0.3)	9.9 (0.9)*	21.2 (1.6)*	∞*	∞*	∞*	∞*	∞*	∞*	68.5 (9.1)*	19.7 (1.4)*	8.2 (1.0)*	5 (0.
ntact perfused dogs (latex tubing; no reservoir)	6	4.3 (0.7)	12.8 (1.4)*	22.0 (0.9)*	∞*	∞*	∞*	∞*	∞*	∞*	43.3 (1.0)*	13.3 (1.0)*	4.7 (0.6)	(

			Perfusion							Perfusion discontinued		
viscerated perfused dogs (hepatic artery patent)	6	5.3[c] (0.2)	5.2[d] (0.2)	16.0 (0.5)*	28.0 (1.9)*	∞*	∞*	∞*	∞*	47.4 (4.7)*	26.4 (3.1)*	12.3 (2.4)*
viscerated perfused dogs (hepatic artery ligated)	6	5.0[c] (0.4)	5.0[d] (0.5)	13.5 (1.6)*	10.3 (0.8)*	8.8 (0.3)*	8.0 (0)*	7.5 (0.6)*	7.5 (0.7)	5.8 (0.3)	5.3 (0.5)	5.0 (0)

alues are mean(±SE). *Indicates paired comparison to control (0 time) values in intact dogs and to post-evisceration ntrol values in eviscerated dogs, $p<0.05$. ∞ = no visible clotting time during 24 hours.

he arterial whole blood clotting time, using a modified Lee and White (25) procedure.

easurements taken prior to beginning perfusion.

re-evisceration control values.

easurements taken prior to beginning perfusion and after evisceration procedure (post-evisceration control).

TABLE 4. Effect of LD_{100} endotoxin on portal vein pressure in eviscerated dogs with and without patent hepatic artery* (Group 4)

Animal No.	Pre-endotoxin		Post-endotoxin[a]	
	MSAP[b]	PVP[c]	MSAP	PVP
		Series 1: Hepatic Artery Patent		
1	135	8	90	18
2	125	3	135	15
3	135	6	125	21
4	150	7	140	16
		Series 2: Heaptic Artery Ligated		
1	160	6	165	2
2	155	6	105	3
3	155	3	155	2
4	160	3	160	3
5	155	4	155	5

*Portal vein ligated in both instances, with all abdominal viscera excluding liver surgically removed.

[a]maximum change in portal vein pressure, 15-30 min post-endotoxin

[b]MSAP = mean systemic arterial pressure (mmHg)

[c]PVP = portal vein pressure (mmHg)

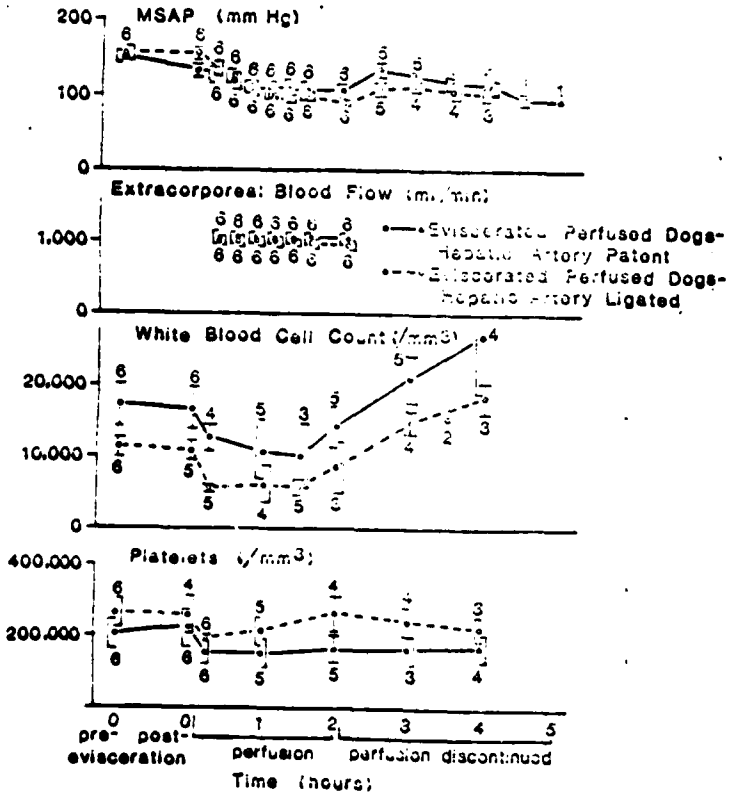

FIGURE 1. Responses of eviscerated dogs during and following extracorporeal perfusion without exogenous anticoagulation (mean±SE); Groups 1 & 3

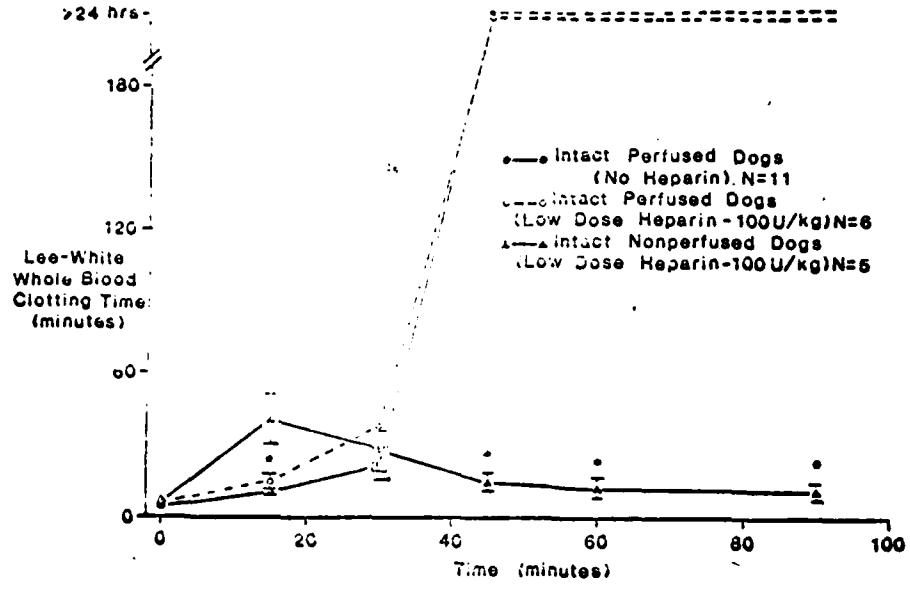

FIGURE 2. Comparison of effects of exogenously administered heparin with endogenously-induced anticoagulation in dogs during extracorporeal perfusion (mean±SE) ; Group 2

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autoanticoagulation, perfusion without anticoagulation, new procedure for perfusion, extracorporeal perfusion, substitutes for exogenous anticoagulation, canine perfusion, improved perfusion, prolonged clotting time, endogenous anticoagulation

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This laboratory has developed a procedure for extracorporeal perfusion without administration of anticoagulants. The present study was designed to determine extra- and intracorporeal requirements for the development of the incoagulable state, to investigate the biologic stability of the perfusion system, and to better define the nature of the hypocoagulable state. Anesthetized dogs were placed on extracorporeal perfusion without exogenous anticoagulation at flows

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exceeding 800 ml/min. Within 45 minutes of perfusion, blood drawn from the animal was incoagulable (clotting time >24 hr), and within 3 hr following discontinuation of perfusion, clotting time returned to normal. Requirements for the autoanticoagulated state were a perfused liver and a pump in the perfusion circuit. Injections of heparin, 100 U/kg, or use of either Tygon or latex tubing in the perfusion circuit, did not modify the time course of the development of the incoagulable state or its reversal following termination of perfusion. Arterial pressure, heart rate, glucose, platelet and fibrinogen concentrations were relatively constant ($p>0.05$); cortisol, white blood cell and neutrophil concentrations increased ($p<0.05$); while the activity of Factors V, VIII and X decreased ($p<0.05$).

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